A CRITICAL EXAMINATION OF STOLL'S METHOD OF COUNTING HOOKWORM EGGS IN FAECES

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Stoll (1923) published a method of estimating the number of hookworm eggs in faeces. The evidence he adduced of the accuracy of his method may be summarised as follows :—

(1) No cultures of the same faeces produced as many larvae as were shown by egg counts.

(2) Soft stools gave less eggs than solid faeces from the same individual.

(3) The first portion passed of a formed stool gave higher counts than random samples of the whole of the same stool. This is explained by the fact that the first portion of a formed stool is drier and hence the eggs are more concentrated in that portion than in later and softer parts of the same stool.

(4) Random samples from the last and softer part of a stool gave slightly lower egg counts than the whole stool mixed.

(5) Counts from day to day from a single individual remained uniform, and this also applied to counts of *Ascaris, Trichuris,* and *Schistosomum* eggs.

(6) Counts from two distinct samples of the same stool are always fairly close together.

Stoll's evidence seems conclusive, but it is at best only circumstantial and the writer found it frequently inaccurate in one particular, viz. : that control cultures always produced fewer larvae than the number of eggs counted in the same faeces. This led to examining the method more closely and testing it in various ways.

Faeces were taken and counts performed in exactly the same way as Stoll describes, and, in addition, small portions (about 200 milligrammes) of the same faeces were accurately weighed in small solid watch glasses and thoroughly mixed with saturated salt solution. Slide after slide was then applied to the surface of the fluid in the watch glasses and all the eggs so obtained counted. This was continued until it was thought that nearly all the eggs had been removed.

Specimen 1.

Counted by Stoll's method. 1st Count, 8. 2nd Count, 10. No. of eggs per gram of faeces average 900.

219 mgr. of faeces mixed with saturated salt solution gave 369+333+162+76 +103+7 eggs on six successive slides.

Total 1,050, which = 4,794 eggs per gram.

202 mgr. of faeces mixed with sat. salt sol. gave 352 + 274 + 251 + 148 + 66 eggs on five successive slides.

Total 1,091, which = 5,391 eggs per gram.

The average number of eggs per gram of two counts by salt concentration was 5,092.

Control cultures produced an average of 4,080 larvae per gram.

Specimen 2.

Counted by Stoll's method. 1st Count, 6. 2nd Count, 6. No. of eggs per gram of faeces average 600.

201 mgr. of faeces mixed with sat. salt sol. gave 9 + 19 + 16 + 11 eggs on four successive slides.

Total 50, which = 274 eggs per gram.

201 mgr. of faeces mixed with sat. salt sol. gave 15 + 9 + 14 + 2 eggs on four successive slides.

Total 40, which = 199 eggs per gram.

The average number of eggs per gram of two counts by salt concentration was 236.5.

Control cultures were not done.

Specimen 3.

Counted by Stoll's method. 1st Count, 2. 2nd Count, 1.

No. of eggs per gram of faeces average 150.

201 mgr. of faeces mixed with sat. salt sol. gave 8 + 9 + 11 + 6 + 10 + 3 eggs on six successive slides.

Total 47, which = 233 eggs per gram.

200 mgr. of faeces mixed with sat. salt sol. gave 13 + 13 + 5 + 9 + 3 + 3 eggs on six successive slides.

Total 46, which = 230 eggs per gram.

The average number of eggs per gram of two counts by salt concentration was 231.5.

Control cultures produced an average of 353 larvae per gram.

Specimen 4.

Counted by Stoll's method. 1st Count, 2. 2nd Count, 1.

No. of eggs per gram of faeces average 150.

200.5 mgr. of faeces mixed with sat. salt sol. gave 8 + 35 + 21 + 7 + 3 eggs on five successive slides.

Total 74, which = 369 eggs per gram.

200.5 mgr. of faeces mixed with sat. salt sol. gave 18 + 15 + 32 + 13 + 8 eggs on five successive slides.

Total 86, which = 429 eggs per gram.

The average number of eggs per gram of two counts by salt concentration was 369.

Control cultures produced an average of 450 larvae per gram.

Specimen 5.

Counted by Stoll's method. 1st Count, 6. 2nd Count, 6.

No. of eggs per gram of faeces average 600.

207 mgr. of faeces mixed with sat. salt sol. gave 27 + 52 + 3 + 2 + 0 on five successive slides.

Total 84, which = 406 eggs per gram.

201 mgr. of faeces mixed with sat. salt sol. gave 24 + 35 + 16 + 2 + 0 on five successive slides.

Total 77, which = 383 eggs per gram.

The average number of eggs per gram of two counts by salt concentration was 394.5.

Control cultures produced an average of 796 larvae per gram.

Specimen 6.

Counted by Stoll's method. 1st Count, 100. 2nd Count, 100.

No. of eggs per gram of faeces average 10,000.

210 mgr. of faeces mixed with sat. salt sol. gave 323 + 319 + 471 + 162 + 72 on five successive slides.

Total 1,347, which = 6,414 eggs per gram.

Control cultures produced an average of 8,833 larvae per gram.

Stoll's method was further checked in the following manner :— Two counts were made by Stoll's method, his instructions being followed in every detail. About ten grams of faeces were placed in a beaker and balanced against an equal weight of water, the two being thoroughly mixed by stirring until the mixture was perfectly homogeneous. Three-gram lots of this mixture were now weighed out

and counted by Stoll's method. It was previously found that faeces are practically of a specific gravity of 1, therefore when mixed with an equal weight of water, a given weight of the mixture will only contain half the amount of faeces that is contained in the same weight of undiluted faeces. In Stoll's method, using undiluted faeces, the number obtained by counting is multiplied by 100 to ascertain the number of eggs in a gram of faeces ; accordingly, if the same weight of the diluted faeces is taken and counted by Stoll's method, it will be necessary to multiply the count by 200 to obtain

Table I

SUMMARY OF ABOVE COUNTS

Number of specimen	Eggs per gram by Stoll's method	Eggs per gram by salt concentration	Larvae per gram in control cultures
1	900	5,092	4,080
2	600	236·5	Not done
3	150	231·5	353
4	150	369	450
5	600	394·5	796
6	10,000	6,414	8,833

the number of eggs per gram in the undiluted faeces, and if Stoll's method is correct these two results should be approximately the same. If the dilution is still more increased by taking part of the faeces and water mixture already made and mixing it with an equal weight of water, a count of this dilution, if counted by Stoll's method, will have to be multiplied by 400 to give the number of eggs per gram in the original faeces. Further dilution in the same proportions will require multiplication by 800.

The above process was carried out with several specimens of faeces and the results are given in Table II. In this table ' first dilution ' means original faeces plus an equal weight of water, ' second dilution ' means part of ' first dilution ' plus an equal weight of water, and ' third dilution ' means a part of ' second dilution ' plus an equal weight of water.

Table II

Number of specimen	Character of Faeces	Undiluted Faeces			First Dilution			Second Dilution			Third Dilution		
		1st Count	2nd Count	Eggs per gram	1st Count	2nd Count	Eggs per gram	1st Count	2nd Count	Eggs per gram	1st Count	2nd Count	Eggs per gram
... ...	Soft, formed... ...	100	117	10,850	69	76	14,500	51	41	18,400	—	—	—
... ... nd count)		—	—	—	78	72	15,000	—	—	—	—	—	—
... ...	Very watery... ...	23	17	2,000	9	8	1,700	—	—	—	—	—	—
... ... nd count)		21	20	2,050	12	12	2,400	—	—	—	—	—	—
... ...	Soft, formed... ...	5	4	450	8	4	1,200	2	2	800	—	—	—
... ...	Liquid	26	41	3,350	15	22	3,700	12	9	4,200	—	—	—
... ...	Soft, formed... ...	53	65	5,900	95	102	19,700	56	64	24,000	—	—	—
... ... nd count)		56	65	6,050	86	95	18,100	41	50	18,200	—	—	—
... ...	Watery, with mucus	9	6	750	3	0	300	1	0	200	—	—	—
... ...	Formed	0	0	0	1	1	200	0	1	200	—	—	—
... ...	Formed, a little softer than 7	1	1	100	0	3	300	0	3	600	0	0	0
... ...	Soft, unformed ...	0	1	50	0	1	100	1	1	400	—	—	—
... ...	Liquid	2	2	200	2	4	600	3	2	1,000	2	1	1,200
... ...	Liquid	11	6	850	7	3	1,000	0	2	400	3	2	2,000

All the weighings and counts were done personally by the writer with the greatest possible care, and accuracy was in no case sacrificed on account of the time taken ; slides were counted twice if there was any doubt that eggs had been missed or counted more than once owing to inaccurate moving of the mechanical stage of the microscope.

The above results obtained by checking Stoll's method against salt concentration and dilution of faeces indicate that the method is not accurate within ten per cent., which is the claim of the originator.

Examination of Table II suggests that the number of eggs obtained by this method varies with the consistency of the original specimen of faeces, higher counts being obtained when the faeces are liquid. The reason for this is difficult to explain, for shaking with glass beads in a solution of deci-normal NaOH for a minute or more, as recommended by Stoll, seems to cause just as complete comminution of the faeces whether they are solid, soft, or liquid to begin with.

REFERENCE

Stoll, N. R. (1923). An Effective method of Counting Hookworm Eggs in Faeces. Investigations on the Control of Hookworm Disease. No. XV. *American Journ. Hygiene*, Vol. III, p. 59.